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MULTI-SOURCE ION FUNNEL

STATEMENT REGARDING FEDERALLY
SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government support under Contract DE-AC0676RLO1830 awarded by the U.S. Department of Energy. The Government has certain rights in the invention.

CROSS-REFERENCE TO RELATED
APPLICATIONS

Not Applicable

BACKGROUND OF THE INVENTION

The growing importance of mass spectrometry for high throughput applications in recent years has stimulated interest in approaches for multiplexing analyses for improving the accuracy of mass spectrometry, and in the development of multiple ion sources. For example, the growing interest in the use of electrospray ionization mass spectrometry (ESI-MS) in proteomic research has virtually created an open-ended demand for high mass measurement accuracy (MMA), where accurate mass measurements for biopolymers can enable their rapid identification and greatly increase the throughput of sample analysis. The results reported by Henry, K. D.; Quinn, J. P.; McLafferty, F. W. *J. Am. Soc. Mass Spectrom.* 1991, 113, 5447-5449; Li, Y. Z.; McIver, R. T.; Hunter, R. L. *Anal. Chem.* 1994, 66, 2077-2083; Kloster, M. B.; Hannis, J. C.; Muddiman, D. C.; Farrell, N. *Biochemistry* 1999, 38, 14731-14737; Zhou, F.; Shui, W.; Lu, Y.; Yang, P.; Guo, Y. *Rapid Commun. Mass Spectrom.* 2002, 16, 505-511; Hannis, J. C.; Muddiman, D. D. *J. Am. Soc. Mass Spectrom.* 2000, 11, 876-883; Flora, J. W.; Hannis, J. C.; Muddiman, D. C. *Anal. Chem.* 2001, 73, 1247-1251; and O'Connor, P. B.; Costello, C. E. *Anal. Chem.* 2000, 72, 5881-5885, among others, have demonstrated that the use of internal calibrants can significantly increase the MMA of MS applications. Traditionally in ESI-MS, internal calibrants have been introduced either simultaneously with the analyte sample solution using a single electrospray, as was shown in Kloster, M. B.; Hannis, J. C.; Muddiman, D. C.; Farrell, N. *Biochemistry* 1999, 38, 14731-14737 or separately using a dual electrospray system as shown in Zhou, F.; Shui, W.; Lu, Y.; Yang, P.; Guo, Y. *Rapid Commun. Mass Spectrom.* 2002, 16, 505-511; Hannis, J. C.; Muddiman, D. D. *J. Am. Soc. Mass Spectrom.* 2000, 11, 876-883; Flora, J. W.; Hannis, J. C.; Muddiman, D. C. *Anal. Chem.* 2001, 73, 1247-1251. In the dual electrospray systems shown in the references given above, the calibrant and the analyte are alternatively introduced through a single capillary, by mechanically switching between the two ion sources. These systems thus suffer from drawbacks associated with mechanical switching, such as relatively slow changes between analyte and calibrant, and difficulties in controlling ion sampling and transmission into the mass spectrometer. Alternatively, with FTICR or other ion-trapping instruments, calibrant ions can be introduced from a second ion source that functions in the vacuum of the MS as described in Winger, B. E.; Castoro, J. A.; Tutko, D.; Campana, J. E. Proceedings of the 44th ASMS Conference, Portland, Oreg., May 1996. Unfortunately, the quality of the calibration obtained from such an arrangement will generally provide lower MMA, since the ions will generally be trapped at somewhat different ion energies and spatially occupy somewhat different regions of the ion trap, which result in "shifts" of mass measurements. Dual electrospray

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may be operated in the continuous mode and sampled simultaneously into the mass spectrometer as described in Zhou, F.; Shui, W.; Lu, Y.; Yang, P.; Guo, Y. *Rapid Commun. Mass Spectrom.* 2002, 16, 505-511. They can also be operated in a sequential mode using mechanical switching devices as described in Hannis, J. C.; Muddiman, D. D. *J. Am. Soc. Mass Spectrom.* 2000, 11, 876-883; Flora, J. W.; Hannis, J. C.; Muddiman, D. C. *Anal. Chem.* 2001, 73, 1247-1251.

Several problems associated with the introduction and use of internal calibrants continue to limit their application. As was reported in Kloster, M. B.; Hannis, J. C.; Muddiman, D. C.; Farrell, N. *Biochemistry* 1999, 38, 14731-14737, when both analyte sample and calibrant are introduced simultaneously into the single electrospray, the ion competition/suppression in the ESI process can significantly reduce sensitivity to the analyte. As was reported in Zhou, F.; Shui, W.; Lu, Y.; Yang, P.; Guo, Y. *Rapid Commun. Mass Spectrom.* 2002, 16, 505-511, when dual electrosprays of analyte and calibrant are operated continuously, the interaction of electrosprays in the ESI interface can also significantly reduce MS sampling efficiency. In addition, as reported in Hannis, J. C.; Muddiman, D. D. *J. Am. Soc. Mass Spectrom.* 2000, 11, 876-883; Flora, J. W.; Hannis, J. C.; Muddiman, D. C. *Anal. Chem.* 2001, 73, 1247-1251, when dual electrosprays are operated in the sequential mode, the limited speed of mechanical switching may significantly decrease the overall MS duty cycle.

Of particular importance for ion trap and FTICR instruments is that calibrant ions contribute to the space charge effect in the MS analyzer and affect the mass measurement accuracy. The introduction of calibrant ions from a different ion source (or at a different time) can also cause the spatial distribution of ions to differ from analyte ions and lead to measurement errors. In addition, the calibrant ions can constitute a significant fraction of the space charge capacity of the ion traps and, thus, constrain the achievable dynamic range of the measurements. These problems become especially pronounced when ESIMS is used for the analysis of a complex biological sample for which extremely high sensitivity and broad dynamic range are required. In designs that provide for a constant rate of calibrant ion introduction (e.g., as for reported dual electrospray designs) and when relative analyte concentration varies widely (e.g., in proteomics), calibrant peak abundances may range from dominant in some spectra to negligible in others, which limits both achievable MMA and dynamic range.

Thus, there remains a need for methods and apparatus that address these issues, and allow effective control of ion transmission into the mass spectrometer. Optimally, such methods and apparatus would allow both static and dynamic control of ion transmission, and would allow modulation of calibrant ion peak intensities while maintaining optimum ion transmission for analyte ions and, thus, provide the basis for simultaneously achieving optimum sensitivity, dynamic range, and MMA.

BRIEF SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to provide a method and apparatus for introducing ions from multiple sources from a region at relatively high pressure to a region at relatively low pressure. It is a further object of the present invention to provide a method and apparatus for introducing ions from multiple sources from a region at relatively high pressure to a region at relatively low pressure in a manner that allows for precise control of the quantity of